*AMINE SYNTHESIS IN RAPIDLY GROWING TISSUES: ORNITHINE DECARBOXYLASE ACTIVITY IN REGENERATING RAT LIVER, CHICK EMBRYO, AND VARIOUS TUMORS**

By Diane Russell† and Solomon H. Snyder‡

DEPARTMENTS OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, AND PSYCHIATRY AND THE BEHAVIORAL SCIENCES, THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE, BALTIMORE

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The amines putrescine, spermidine, and spermine occur in considerable quantities in most animal and plant tissues examined. These compounds stabilize ribosomes and nucleic acids and affect nucleic acid synthesis, possibly by an interaction of the amine groupings with acid moieties of the nucleic acids.[1] In rapidly growing tissues such as regenerating rat liver and chick embryo, the concentration of spermidine increases at a rate that parallels the increase in RNA concentration.[2–5] After hypophysectomy, there is a marked decrease in the spermidine content of rat liver that can be restored to normal levels by the administration of growth hormone.[6]

The activity of histidine decarboxylase, which forms histamine, a diamine, is markedly increased in some rapidly growing tissues[7, 8] but not in others.[9, 10]

Ornithine decarboxylase is an enzyme that forms putrescine, the immediate precursor of spermidine, in bacteria[11] and mammals[12] and may catalyze a rate-limiting step in polyamine synthesis. In the present study, we have examined the activities of ornithine decarboxylase, histidine decarboxylase, and other amino acid decarboxylases in regenerating rat liver, chick embryo, and several rat tumors.

Materials and Methods.—D,L-ornithine-1-C^{14}-monohydrochloride (2.3 mc/mmole), D,L-ornithine-5-C^{14}-hydrochloride (8.3 mc/mmole), D,L-lysine-1-C^{14}-monohydrochloride (3.7 mc/mmole), L-histidine-carboxyl-C^{14} (13.7 mc/mmole), D,L-phenylalanine-1-C^{14} (2.8 mc/mmole), D,L-leucine-1-C^{14} (7.3 mc/mmole), D,L-methionine-carboxyl-C^{14} (3.4 mc/mmole) were obtained from the New England Nuclear Corp. NSD-1055 (4-bromo-3-hydroxybenzyloxamine-dihydrogen phosphate) was generously donated by Dr. David J. Drain of Smith and Nephew Research, Ltd.

Sprague-Dawley female rats (150–200 gm) were lightly anesthetized with ether. Partial hepatectomy was performed by the method of Higgins and Anderson.[13] Sham-operated animals were anesthetized and laparotomized. Rats were killed by decapitation under light ether anesthesia, and the liver remnants (caudate and right lateral lobes) were removed, chilled on ice, and homogenized in 5 vol of 0.05 *M* sodium-potassium phosphate buffer, pH 7.2. Unless otherwise specified, the homogenate was centrifuged at 20,000 × *g* for 20 min and the supernatant fluid was used for enzyme assay.

Assay for amino acid decarboxylase activity: Enzyme activity was determined by measuring the liberation of $C^{14}O_2$ from carboxyl-labeled substrate. Incubations were carried out in a 25-ml Erlenmeyer flask equipped with a rubber stopper supporting a polyethylene center well (Kontes Glass Co., no. 88230) that contained 0.3 ml of a 2:1 mixture of ethanolamine and 2-methoxyethanol. Incubation mixtures consisted of 0.1 μmole of pyridoxal phosphate, 0.2–0.8 ml of enzyme preparation, 0.5 μc of carboxyl-labeled amino acid, and 0.05 *M* sodium-potassium phosphate buffer, pH 7.2, to make a final volume of 2 ml. All components of the system except for substrate were agitated for 10 min at 37° prior to the addition of substrate, and the incubation was continued for 30 min at 37°. The reaction was stopped by injecting 1.0 ml of 2.0 *M* citric acid into the reaction

mixture through the rubber stopper. The mixture was agitated for an additional 30 min at 25° to allow complete absorption of the evolved $C^{14}O_2$. The center well was removed, placed in a vial containing 2 ml of ethanol and 10 ml of toluene phosphor (0.4% 2,4-diphenyloxazole and 0.01% β-bis-(2-phenyloxazole) benzene in toluene), and assayed for radioactivity in a liquid scintillation spectrometer. All values were corrected for a heated enzyme blank. Enzyme activity was linear for the duration of the incubation period.

Ornithine decarboxylase activity was also assayed by measuring the C^{14}-putrescine formed when D,L-ornithine-5-C^{14} was incubated with tissue extracts. Incubation conditions were the same as above except that glass-stoppered centrifuge tubes were used instead of Erlenmeyer flasks. The reaction was stopped with 1.0 ml of 0.1 *N* NaOH, the solution was saturated with sodium chloride, and the amine product was extracted into 7 ml of 1-butanol. The organic phase was washed once with 1 ml of 0.1 *N* NaOH, after which 1 ml of the organic phase was transferred to a vial and the radioactivity determined after the addition of 2 ml of ethanol and 10 ml of toluene phosphor. In some experiments, the butanol extract was evaporated to a small volume and chromatographed on paper in two systems (1-propanol:NH_4OH, 3:1; 1-propanol:conc. HCl:H_2O, 3:1:1). In both systems, there was a single peak of radioactivity that corresponded to authentic putrescine.

Tumors: Multiple-deviation Morris hepatomas were generously donated by Dr. H. P. Morris. These tumors were rapidly growing and poorly differentiated, with an abnormal chromosome complement.[14]

Three sarcomas examined in this study were generously donated by Drs. C. A. Reznikoff and G. E. Gey.[15] The STAT-1 tumor was obtained by inoculating rats with tumor cells produced by treating a tissue-culture line of normal rat fibroblasts for 3 days with DNA from the Walker 319 fibrosarcoma. The STAT-1 tumors, which were harvested 11 days after inoculation, were poorly differentiated sarcomas with hypotetraploid chromosome numbers, many fragments, and breaks. The STAT-3 tumor resulted from the inoculation of rats with fibroblasts treated for 30 days with DNA from a Walker 319 fibrosarcoma and was harvested 30 days after inoculation. This tumor was poorly differentiated with a quasi-diploid chromosome number.

The NRF-TFF sarcoma was harvested from rats 30 days after inoculation with fibroblasts that had become spontaneously transformed in tissue culture from cultured rat fibroblasts that had been frozen and thawed twice. It was poorly differentiated with bloody ascites and hypotetraploid chromosome number.

Chick embryos incubated at 60% humidity and 37.2–37.4°C were obtained from Truslow Farms, Chestertown, Maryland, at all stages of development from 2-day embryos to 1-day-old chicks.

Results.—Effect of partial hepatectomy on hepatic ornithine decarboxylase activity: Rats were hepatectomized or subjected to sham operation and killed 4, 16, or 96 hours after operation. Liver remnants were assayed for ornithine decarboxylase by measuring both $C^{14}O_2$ production and C^{14}-putrescine formation (Table 1). At all times, ornithine decarboxylase activity was the same whether measured by the formation of C^{14}-putrescine or the liberation of $C^{14}O_2$. Four hours after partial hepatectomy, enzyme activity was almost ten times as great as control levels. After 16 hours, it was 17 times greater in liver remnants from partially hepatectomized rats than in control liver. After 96 hours, enzyme activity was decreased but was still triple the control values.

The substrate concentration used (50 μM as L-ornithine) was nonsaturating. However, in some experiments, excess ornithine (2 mM) was used as substrate, and the same changes in enzyme activity were obtained after partial hepatectomy.

TABLE 1. *Stoichiometry of the decarboxylation of ornithine and the formation of putrescine.*

Hours after operation	C^{14}-Putrescine Synthesis (mμmoles/30 min/gm ± SEM)		$C^{14}O_2$ Production (mμmoles/30 min/gm ± SEM)	
	Hepatectomized	Sham-operated	Hepatectomized	Sham-operated
4	35.2 ± 2.8*	3.7 ± 0.5	38.9 ± 3.9*	3.2 ± 0.3
16	68.7 ± 6.2†	4.1 ± 0.8	75.0 ± 6.9†	4.1 ± 0.3
96	9.6 ± 0.6‡	3.6 ± 0.4	12.3 ± 0.2‡	3.8 ± 0.7

Groups of five to ten rats were partially hepatectomized or sham-operated and killed at varying time intervals. Livers were assayed for ornithine decarboxylase by measuring the formation of C^{14}-putrescine from D,L-ornithine 5-C^{14} or the evolution of $C^{14}O_2$ from D,L-ornithine-1-C^{14}.

* Differs from sham-operated control value ($P < 0.001$).

† Differs from both sham-operated control value ($P < 0.001$) and 4-hr partially hepatectomized value ($P < 0.005$).

‡ Differs from sham-operated control value ($P < 0.05$), 4-hr partially hepatectomized value ($P < 0.001$), and 16-hr partially hepatectomized value ($P < 0.001$).

Ornithine decarboxylase activity in supernatant preparations from 24-hour hepatectomized and sham-operated control rat livers was unaffected by dialysis for 24 hours against H_2O or by the addition of Mg^{++}, Ca^{++}, PO_4^{---}, SO_4^{---}, and HCO_3^-. Enzyme activity was the same whether assayed in 10,000 × g or 100,000 × g supernatant fractions.

In order to examine whether the process of liver regeneration affected ornithine decarboxylase activity in extrahepatic tissues, the following tissues were assayed for enzyme activity in rats 16 hours after partial hepatectomy or sham operation: pancreas, spleen, stomach, adrenals, and kidneys. Enzyme activity in these tissues ranged between 5 and 15 mμc of $C^{14}O_2$/30 min/gm and was unaltered by hepatectomy.

To examine the time course of changes in ornithine decarboxylase activity after partial hepatectomy, groups of 5–10 rats were killed at 11 time intervals between 1 and 96 hours after operation (Fig. 1). One hour after operation, enzyme activity was tripled. Peak activity 22 times greater than control levels was observed 16 hours after operation. Enzyme activity decreased gradually but was still more than triple the control values after 96 hours.

In an experiment designed to detect the presence of an inhibitor of ornithine decarboxylase in livers of control rats, enzyme preparations from control and four-hour regenerating rat livers were mixed together and assayed for ornithine decarboxylase activity. Enzyme activity was additive in all cases, indicating the absence of either inhibitors or activators in livers from control or hepatectomized rats.

In order to determine whether other amino acid decarboxylases were affected by partial hepatectomy, groups of five rats were hepatectomized or sham-operated and killed after four hours. Their livers were assayed for the evolution of $C^{14}O_2$ after incubation with the following carboxyl-labeled amino acids: ornithine, histidine, lysine, phenylalanine, methionine, and leucine (Fig. 2). Ornithine decarboxylase activity in hepatectomized rats was nine times greater than in sham-operated animals. Enzymatic decarboxylation of the other amino acids was the same in sham-operated and in hepatectomized rats. The high level of phenylalanine decarboxylation was presumably produced by nonspecific aromatic L-amino acid decarboxylase, which is very active in normal liver.[16]

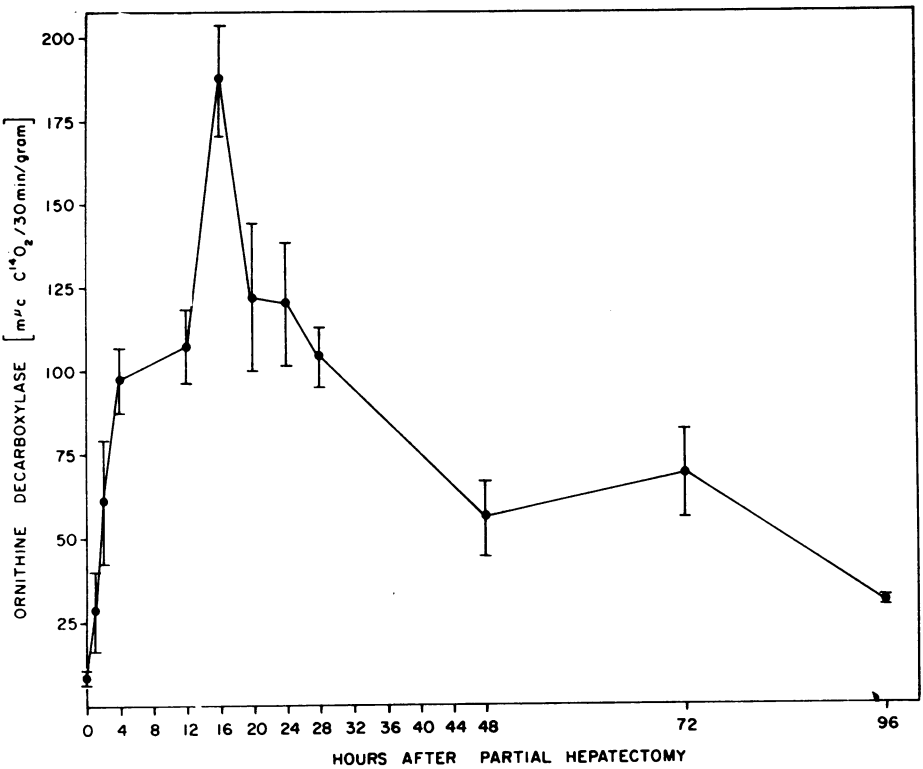

FIG. 1.—Time course of increase in ornithine decarboxylase activity in the rat liver remaining after partial hepatectomy. Each point represents the mean ±SEM of five to ten rats.

Ornithine decarboxylase activity during chick embryo development: Chick embryos at varying stages of development were assayed for ornithine, lysine, and histidine decarboxylase activity. The activities of histidine and lysine decarboxylases were very low at all stages examined, with no variations among the different stages. In marked contrast, considerable ornithine decarboxylase activity was detected in three-day embryos, although unfertilized eggs had negligible activity (Fig. 3). Enzyme activity tripled between three and four days, attained a peak level at five days, and fell to less than 25 per cent of peak values by seven days. The five-day level of ornithine decarboxylase activity was three times greater than the highest values observed in regenerating liver. Thereafter enzyme activity gradually declined to negligible levels by 20 days. In embryos at 11-, 12-, 13-, 16-, and 19-day stages and in chicks 1 day after hatching, the following individual tissues were assayed for ornithine decarboxylase activity: liver, heart, stomach, brain, muscle, skin, eye, gizzard, and pancreas. Negligible activity was detected in all tissues of the 19-day embryo and the 1-day-old chick. At the other stages, enzyme activity was elevated in all tissues except the eye. Highest activity was detected in skin and muscle.

Ornithine and histidine decarboxylase activity in tumors and fetal rat liver: Adult rat liver, 20-day fetal liver, and several tumors were assayed for ornithine

and histidine decarboxylase activity (Fig. 4). Enzymatic activity for both was very low in adult rat liver. In the other tissues examined, there was an inverse relationship between the activity of histidine decarboxylase and ornithine decarboxylase. In fetal rat liver, extremely high histidine decarboxylase activity was detected, whereas ornithine decarboxylase activity was quite low. This con-

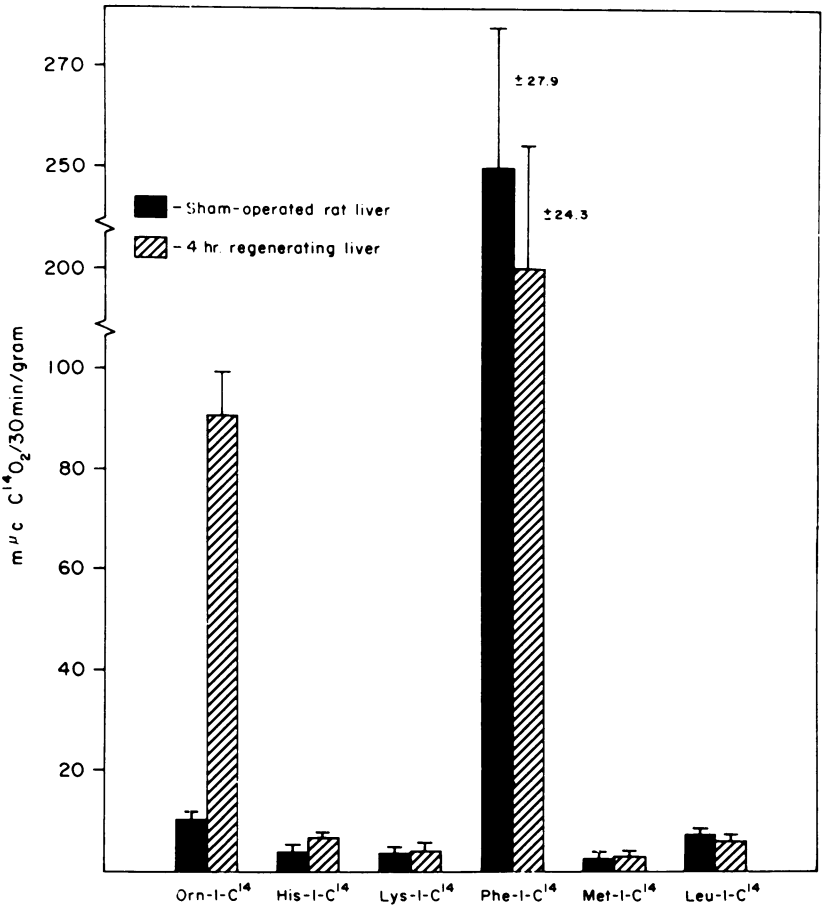

FIG. 2.—Activity of amino acid decarboxylases in regenerating rat liver. Vertical bars indicate the SEM for groups of five to ten rats.

trasts dramatically with the high ornithine decarboxylase but low histidine decarboxylase[10] activity in regenerating rat liver. Hepatomas, STAT-3 sarcoma, and XRF-TFF sarcoma showed high histidine decarboxylase activity and low ornithine decarboxylase activity. In STAT-1 sarcoma, on the other hand, ornithine decarboxylase activity levels were extremely high, comparable to levels observed in regenerating rat liver, whereas histidine decarboxylase activity was quite low.

Discussion.—The dramatic increase in the activity of ornithine decarboxylase that we observed in regenerating rat liver was specific for the decarboxylation of ornithine. Out of a large number of enzymes that have been surveyed in regenerating liver,[4] ornithine decarboxylase shows the earliest change. The peak of ornithine decarboxylase stimulation at 16 hours precedes peak increases in DNA and RNA synthesis.[17] Church and McCarthy[18] have observed the synthesis of a new RNA species in mouse liver as early as one hour after partial hepatectomy.

The biosynthesis of polyamines in bacteria involves the decarboxylation of ornithine to form putrescine, which is then linked to a propylamine moiety derived from the enzymatic decarboxylation of S-adenosylmethionine. There is evidence that a similar pathway is present in mammals.[11, 12, 19] In regenerating liver, putrescine concentration doubles 4 hours after hepatectomy and remains at this level for 72 hours, whereas increases in spermidine concentration are not detectable until 24 hours and peak 72 hours postoperatively at levels about twice control values.[20, 21] Early increases *in vivo* in C^{14}-putrescine formation observed in regenerating liver after injection of C^{14}-ornithine or C^{14}-methionine[21] suggest that the early stimulation of ornithine decarboxylase may be responsible for the changes in polyamine synthesis, although hepatic S-adenosylmethionine decarboxylase[19] may play a role.

In the chick embryo, there was a striking increase in ornithine decarboxylase activity, which peaked at five days of development and declined thereafter to negligible levels by hatching. In a parallel study of polyamine and nucleic acid concentrations, Caldarera[3] found an increase of putrescine that peaked at 5 days,

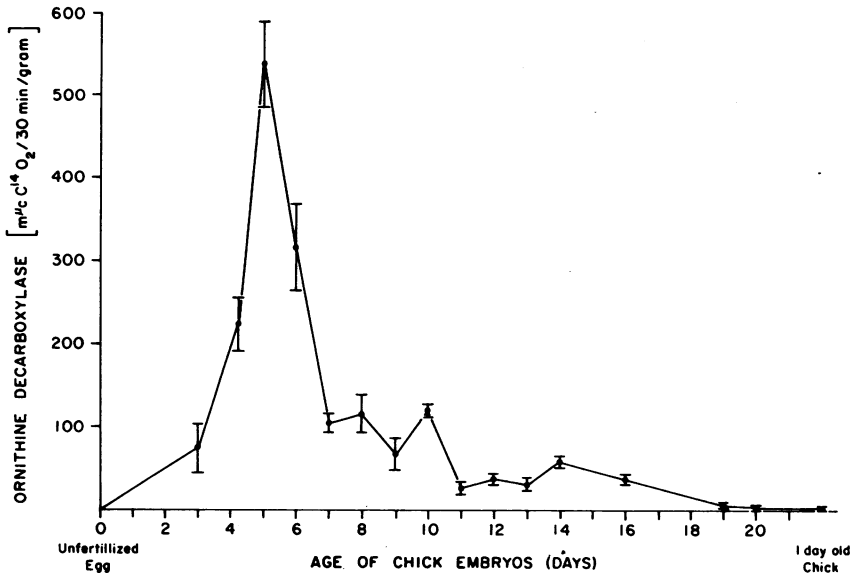

FIG. 3.—Changes in ornithine decarboxylase activity during the development of the chick embryo. Each point represents the mean ±SEM for at least two pools of embryos (5–36 embryos per pool).

whereas spermidine concentration attained peak levels at 7 days; RNA and DNA content also peaked at 7 days, whereas the RNA/DNA ratio was highest at 14 days. As in regenerating liver, this early increase of putrescine raises the possibility that ornithine decarboxylase activity regulates polyamine synthesis, and enhancement of its activity precedes increases in nucleic acids.

In the STAT-1 sarcoma, ornithine decarboxylase activity of great magnitude was observed, higher than in any tissues examined except for regenerating liver and chick embryo; histidine decarboxylase activity of this tumor was quite low. In marked contrast, histidine decarboxylase activity was greatly elevated in the Morris hepatoma, NRF-TFF sarcoma, and STAT-3 sarcoma, whereas the ornithine decarboxylase activity of those tumors was considerably lower. The inverse relationship between ornithine decarboxylase and histidine decarboxylase activity in these tumors suggests that whatever role the polyamines play in rapid tissue growth, it may also be assumed in some tissues by histamine. This hypothesis is supported by the high histidine decarboxylase activity and low ornithine decarboxylase activity in fetal rat liver on the one hand, and the elevated ornithine decarboxylase activity and low histidine decarboxylase activity in chick embryo on the other hand. Putrescine, the product of ornithine decarboxylase activity, is a diamine, as is histamine, and may also have an important function in rapidly growing tissues.

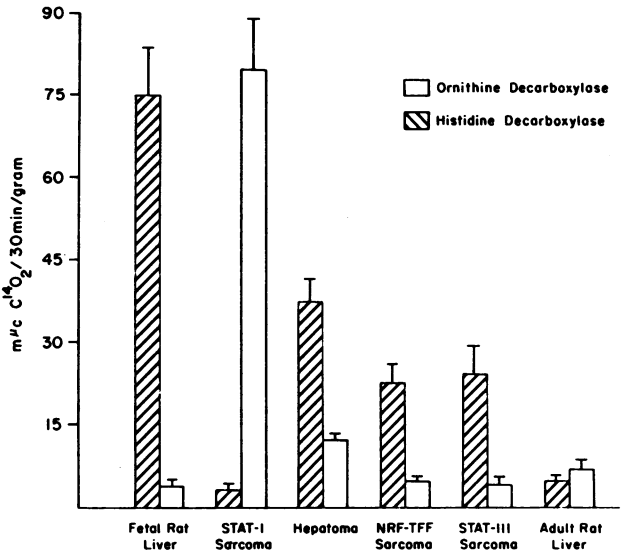

FIG. 4.—Ornithine decarboxylase and histidine decarboxylase activity in fetal and adult rat liver and in rat tumors. Tumor columns represent the mean and SEM for two to three animals. The liver data represent the mean and SEM for five to ten animals.

Polyamines affect ribosomes and nucleic acids and occur ubiquitously in living organisms. However, no major physiological role has yet been clearly defined

for these compounds. Our findings, taken together with those of other investigators,[2, 3, 20, 21] indicate that polyamines and diamines may have important functions in rapidly growing tissues. The very early and striking increase in ornithine decarboxylase activity in regenerating rat liver and chick embryo, preceding increases in nucleic acids, suggests that such a function has to do with the initiation of the rapid growth process.

Summary.—The activity of ornithine decarboxylase is dramatically elevated in regenerating rat liver; enzyme activity triples 1 hour after partial hepatectomy and is 25 times control levels after 16 hours. In chick embryos, high levels of ornithine decarboxylase activity are present in 3- to 16-day embryos, after which enzyme activity decreases rapidly to negligible levels in 1-day-old chicks. High ornithine or histidine decarboxylase activity occurs in certain sarcomas and hepatomas.

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